Plant Physiol. (1969) 44, 821–825

Action of Phytochrome During Prechilling of Amaranthus retroflexus L. Seeds

R. B. Taylorson and S. B. Hendricks

Crops Research Division, United States Department of Agriculture, Beltsville, Maryland 20705
and United States Department of Agriculture, Mineral Nutrition Laboratory,
Plant Industry Station, Beltsville, Maryland 20705

Received December 19, 1968.

Abstract. Dark germination of *Amaranthus retroflexus* L. seeds at 35° increased after several days of prechilling at 20° or lower. Irradiation with far-red light for short periods during the early hours of a prechilling period at 10° inhibited subsequent dark germination at 35°. The inhibition was completely reversible with red light. Far-red irradiation in the latter part of the prechilling period was less effective. Increased dark germination of *A. retroflexus* seeds following a prechilling period at 20° or less is attributed to action of pre-existent P_{FR}, the far-red absorbing form of phytochrome, within the seeds. Inactivation of P_{FR} was found to proceed *ca.* 4 times more rapidly at 25° than at 20°. Failure of imbibition temperatures above 20° to increase dark germination of *A. retroflexus* seeds is attributed to the rapid thermal reversion of pre-existent P_{FR}. We suggest that the action of prechilling (layering) on many other seed kinds arises in a similar way.

Light sensitive seeds either require light to initiate germination, or to prevent it. Action of the far-red absorbing form of phytochrome, P_{FR}, is known to be the light-dependent factor for the former. We are concerned here with P_{FR} action on seeds of *Amaranthus retroflexus* L. as a function of temperature and duration of treatments. The several observations bear both on the manner of phytochrome action and on some aspects of what is variously known as stratification, layering, prechilling, or low-temperature after-ripening of seeds.

Although the enhancement of germination of seeds of many species by initially incubating for a period at low temperatures has been vastly documented (3,8) since publication of Elvyn's "*Discourse of forest trees*" in 1664, a beginning is only recently being made toward an understanding of the processes involved. Pertinent observations with respect to the functioning of P in such phenomena were made by Toole *et al.* (10) and by Scheibe and Lang (7). The discovery of P by Borthwick *et al.* depended in part on the promotion of germination through the presence of some pre-existent P_{FR} (4, 5) in lettuce seed that had imbibed water in darkness. When the seed were held first at 35° in darkness, this pre-existent P_{FR} reverted to the red absorbing form of phytochrome (P_R) sufficiently to reduce subsequent germination at 20°. Study of the lettuce seed responses led Scheibe and Lang to conclude that "the promotive effect of low temperature on dark germination is most probably a result of prevention or delay of transformation of physiologically active phytochrome to an inactive form." We further this conclusion.

Materials and Methods

Mature *A. retroflexus* seeds (designated as lot No. 46) were collected near Beltsville, Maryland during August 1966, and placed in storage at −10° in sealed polyethylene containers. Their moisture content was 10 %. Other similarly collected and stored seeds were used to verify results obtained with lot No. 46. Seeds were germinated in 9-cm petri dishes with 2 discs of Whatman No. 3 filter paper moistened to a shiny appearance with tap water. After planting, the dishes were immediately enclosed in light-tight, black cloth bags, and placed in dark cabinets maintained at ± 1° of the appropriate temperature.

Red light (R) was obtained by filtering the light from a bank of 18, 96-inch slimline T8 cool white fluorescent tubes through 2 layers of red cellophane. Dishes were placed 1 m from the source which at this distance gave 0.6 mw/cm² of 600 to 680 nm radiation. Far-red (FR) irradiations were provided by three 300-w incandescent filament lamps filtered by 2 layers each of red and blue cellophane and 2 inches of water. Intensity of this source at 1 m was 0.75 mw/cm² in the 700 to 750 nm region. Energy distributions from these sources are given in reference (1).

Immediately after irradiation, seeds were returned to their bags and the respective temperature cabinets Germination at 35° was counted after 3 days. Germination percentages are the average of duplicate lots of 100 seeds from at least 2 separate experiments.

Results

Background. The seeds germinated only 5 % or less in darkness over a range of constant and alternating temperatures. However, after *ca.* 3 days of dark imbibition at 35°, complete germination was attained after 5 min R-exposures and 3 further days in darkness at 35°. Analysis of the time-temperature relationships influencing germination of the seeds in response to brief exposures to R revealed that although imbibition temperature was not critical, the seeds had to be at 35° (or higher) to germinate (table I). Germination at 30° was variable, and below 30° was consistently low, even with prolonged germination periods. Subsequent mention of germination implies that it was conducted for 3 days at 35° after the indicated pretreatments.

Table I. *Effect of Imbibition Vs. Germination Temperature on Response of* A. retroflexus *Seeds to Red Light (R)*

The seeds were held for 3 days at each temperature.

Pre-irradiation imbibition	Post-irradiation germination	Light treatment	
		None	5 Min R
temp	*temp*	*% germination*	
20	20	...	1
"	25	...	2
"	30	...	8
"	35	6	92
35	20	...	1
"	25	...	2
"	30	...	3
"	35	3	95
25	25	...	2
30	30	...	3

Time of dark imbibition required to allow maximum germination with R treatment varied with imbibition temperature. At 35°, 36 hr was sufficient, while more than 9 days was required at 5°. Three days imbibition at any temperature between 5° and 35° allowed at least 50 % germination after brief R exposures. The involvement of phytochrome in the R promotion of germination of these seeds, was clearly established by FR reversibility of R potentiated germination. Further details of the promotion and inhibition of these seeds by light will be reported elsewhere.

Influence of Prechilling. The prechilling effect was displayed as increased dark germination of seeds previously imbibed in darkness for various times at temperatures of 20° or lower (table II). Similar pretreatments at 25° and above did not lead to increases. Prechilling at 10° and 15° produced nearly equal effects over periods longer than 1 day. A 28-day prechilling period at 10° led to 80 % germination. Dark germination was found to increase linearly as duration of prechilling at 10° was extended from 3 to 28 days and, by linear extrapolation, would be complete after *ca.* 40 days.

Table II. *Typical Effects of Prechilling for Several Time-Temperature Combinations on Subsequent Dark Germination of* A. retroflexus

Prechill temp	Prechill — days						
	0	1	3	6	9	12	15
	% Germination at 35°						
5	3	1	9	24	30	38	30
10	3	1	29	48	40	54	53
15	3	10	27	35	47	56	68
20	3	15	12	35	28	27	26

Effect of Far-red During Prechilling. The involvement of P_{FR} during prechilling of seeds was first detected as a result of 5 min FR irradiations during prechilling. After exposure, the seeds were returned to 10° for the remainder of the 6-day prechilling period before transferring to 35°. Single FR-irradiations during the interval of 24 to 72 hr of prechilling gave maximum suppression of subsequent dark germination (Fig. 1-A). Two FR-irradiations, one given during, and one at the end of the prechilling period, were less effective than a single irradiation in the optimum 24- to 72-hr period.

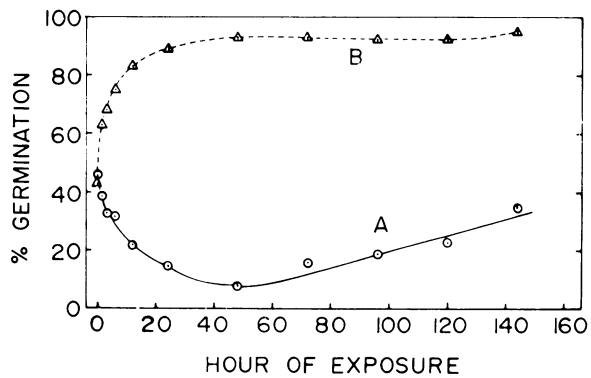

FIG. 1. Effects of 5 min FR (curve A) and R (curve B) irradiations after various hours in a 144 hr 10° prechilling of *Amaranthus retroflexus* seeds on subsequent dark germination at 35°.

Irradiation with 5 min R at or after 24 hr of a 6-day, 10° prechilling period resulted in nearly complete promotion when the seeds were subsequently germinated at 35° (Fig. 1-B). The FR inhibition and R promotion were reversible, in accordance with typical phytochrome control. The resultant germination depended on the nature of the final irradiation, and hour of prechilling when it was applied.

Rate of P_{FR} Action. The gradual decrease in effectiveness of FR during the final hours of prechilling (Fig. 1-A) suggested that increasing numbers of the seeds had completed their requirement for P_{FR} before its removal by photoconversion to P_R.

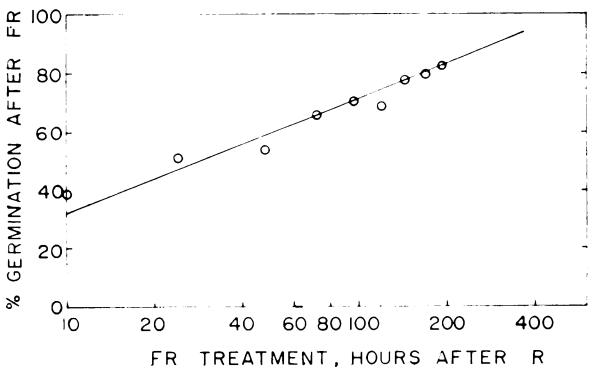

FIG. 2. Rate of P_{FR} action on a stage of *Amaranthus retroflexus* seed germination at 10°. Note text for explanation.

The rate of P_{FR} action at 10° was measured in another experiment (Fig. 2). Seeds were irradiated for 5 min with FR after 48 hr imbibition at 10° to minimize changes in dark germination. They were then held for an additional 72 hr at 10° to insure high levels of promotion by a 5-min/R-irradiation at the end of this period. Some seeds were then given 5-min/FR-irradiations. Others were irradiated with FR at subsequent 24-hr intervals. Immediately after each FR exposure, the lot was transferred to 35° in darkness for germination. The various lots of seeds were exposed to 10° for periods from 0 to 192 hr following R- and prior to FR-irradiation. The results (Fig. 2) showed that germination increased in an essentially exponential manner from 39 % at the 0 hr to about 80 % at the 192 hr. In similar experiments (results not shown), seeds held at 20° after R-irradiation reached the 80 % germination level after 48 hr. The time required for completion of reactions in which P_{FR} participates decreased about 4-fold for each 10° increases in temperature.

Thermal Inactivation of P_{FR}. We can now ask why the prechilling response is not evident above 20°. A possible explanation is that the pre-existing P_{FR} is inactivated rapidly at the higher temperatures, or at least that inactivation proceeds faster than does the germination process. Because the seeds require a 35° temperature to express germination, it was possible to devise experiments to measure apparent dark inactivation of P_{FR} at temperatures below 35°. This was accomplished by imbibing seeds for several days at various temperatures, irradiating with saturating levels of R, and then returning the seeds to darkness at the desired temperatures. At various intervals, the seeds were transferred in darkness to 35°. The resultant germination was taken as an integrated measure of dark inactivation of the P_{FR} and the progress of the seed toward germination. Results at 20° and 25° (Fig. 3) indicated that the rate of change in response was approximately 4 times faster at 25° than at 20°. Extrapolating these data to 30° and 35° (assuming

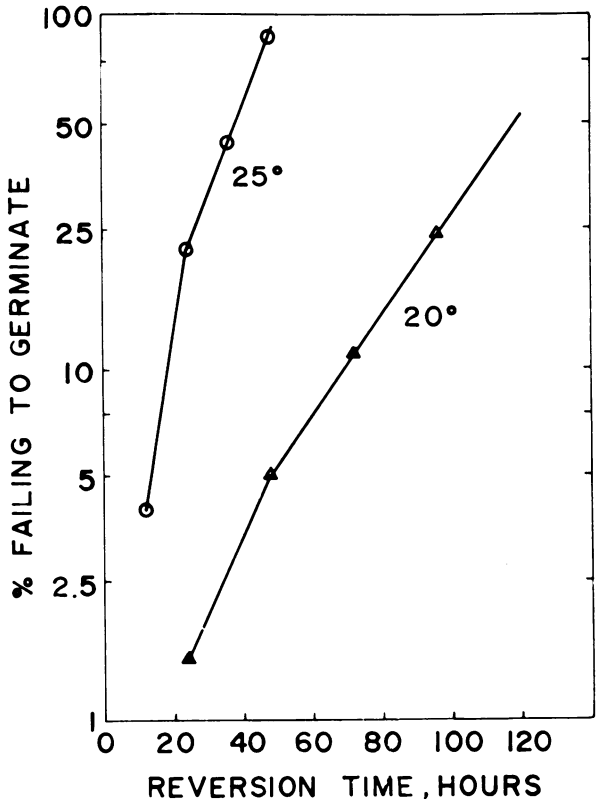

FIG. 3. Rate of apparent dark reversion of P_{FR} in *Amaranthus retroflexus* seeds at 20 and 25°. Note the text for explanation.

the same increase in rate per 5° increase) indicated that at 35° the change would be essentially complete within *ca.* 6 hr. At 10°, the rate of change was not measurable over a 144-hr period. Attempted measurements at 30° failed because of some germination.

If a prechilling period is preceded by a 35° pretreatment (for the pre-existent P_{FR} to be thermally

Table III. *Effect of Various Periods of High Temperature Pretreatment (35°) on the Dark Germination of* A. retroflexus *Following a 6 Day Prechilling Period*

Treatment — hr At 35°	10°	% Germination at 35°
0	144	49
2	142	40
4	140	41
8	136	17
12	132	8
24	120	5
48	96	3
72	72	4
0	120	44
0	96	43
0	72	38

inactivated instead of removed by FR-photoreversion), the effects of prechilling on subsequent germination were negated (table III). The marked break in response is between 4 and 8 hr, which is in agreement with the extrapolation of the preceding experiment. If the seeds are held at 10° for 24 hr to permit full expression of pre-existent P_{FR} prior to placing at 35°, only 12 hr is required for suppression to 3 to 5 % germination and 6 hr is almost enough.

Discussion

The subject on which our experiments bear is the extent to which low temperature effects on seed germination depend on the presence of P_{FR}. The chief matters to disentangle are the extents to which P_{FR} might be inactivated, or revert to P_R at a particular temperature, the degree of progress towards germination, and possible confusion with non-phytochrome requiring processes. Previous work (2, 6, 7, 10) has been on lettuce seeds which germinate in darkness at low temperatures (5°–10°), require light at intermediate temperatures (20°–30°), and fail to germinate at 35°. Ikuma and Thimann (2) concluded that: "Low temperature (2°) can substitute for red light causing full germination on subsequent transfer to 25°. However, this effect of low temperature is not reversed by far-red, so that, like gibberellin, it must act at a point other than that controlled by phytochrome." Scheibe and Lang (7) showed that some of these effects arose from the residual amount of P_{FR} remaining after exposure to far-red radiation. Roth-Bejerano *et al.* (6) discussed possible production of some type of cofactor at 37° for P_{FR} action as an alternative to explain the promotive effects on germination at low temperatures of repeated far-red exposure at high temperatures. Our findings with *A. retroflexus* support and extend Lang and Scheibe's (7) results with lettuce.

A. retroflexus seeds germinate best at high temperatures (*ca.* 35°), whether or not the particular lot requires light for germination. The prechilling effects on these seeds represents typical low temperature effects sometimes described as stratification, *etc.* Such responses are not atypical among other lots of *A. retroflexus* seeds. Several other lots, which differed in year or location of collection, behaved similarly to the lot used in these studies in that they did not germinate well in darkness, but did after R-irradiation and displayed increased dark germination at high temperatures following prechilling.

In the particular lot used in these studies, the increased dark germination following prechilling could be traced to the action of pre-existent P_{FR}. If imbibition was at temperatures above 20°, essentially no dark germination occurred. This is attributed to a rapid, thermal inactivation of the pre-existent P_{FR} without the seeds preceding adequately towards a display of germination. Physiological stages were evident in the low temperature (10°) transformation of seeds to the dark germinating condition. During the first 48 hr of prechilling, the apparent reconstitution (rehydration) of pre-existent P_{FR} within the seeds occurred. Evidence for this release is based on the increasing effectiveness of single FR irradiations from the beginning of a 10° imbibition to 48 hr later (Fig. 1-A). The reconstitution was readily detectable within 3 hr after imbibition commenced. Surprisingly, perhaps, 3 hr of imbibition at 10° coincides with an uptake of only *ca.* 2 % water from fresh weight basis. Rapid appearance of photoreversible phytochrome has also been observed in *Pinus palustris* seeds and embryos on addition of water (9). Apparently, P_R in *A. retroflexus* underwent a similar reconstitution, since single saturating R-irradiations promoted increasing numbers of seeds until *ca.* 48 hr of 10° imbibition has elapsed (Fig. 1-B). Substantial promotion was obtained with R given 1.5 hr after imbibition commenced. These data indicate that phytochrome will undergo transformation in nearly air-dry *A. retroflexus* seeds. It is evident, however, that these changes are only detectable after a period of prechilling.

A final stage, that of completion of a part of the germination reactions in which P_{FR} participates, became prominent 96 hr after imbibition started and continued at a slow rate for the remainder of the 144-hr prechilling period (Fig. 1-A). During this stage, FR becomes less effective in inhibiting subsequent dark germination at 35° as time progressed, probably because of reaching some adequate level of action in particular seeds. This stage apparently continues at 10° so long as P_{FR} remains inasmuch as longer periods of prechilling gave increasingly larger percentages of dark germinating seeds (table II).

The germination process of *A. retroflexus* appears to have several phases in that the promotion of dark germination at 35° is induced by prechilling at 10°, but does not cause germination at 10° over long periods. The prechilling phase depends on the presence of P_{FR} for a considerable period. Its absence at 20° and higher apparently is a result of rapid inactivation on P_{FR} to P_R relative to the rate of action.

An estimate of the temperature coefficient for the rate of P_{FR} reversion to P_R is based on the results shown in Fig. 3. The 2 curves indicate germination in response to a saturating R-irradiation given after imbibition at 20° and just prior to holding for various hours at 20° or 25°, respectively. The curves are simple exponentials within limits of error. The ratio of hr required to reduce germination to a given percentage (abscissa) upon return to 35° is taken a measure of the relative times to reach the same P_{FR} level at the 2 temperatures. The ratio is about

4 to 1, that is, the rate of apparent reversion is 4 times greater at 25° than at 20°. While this is seemingly high, it is not unreasonable either on a kinetic basis, involving, as it does, the interaction of a protein and a chromophore, or on a basis of physiological display. The latter indicates that the rate of reversion at 10° is exceedingly slow compared with the rate at 35°.

The rate of P_{FR} to P_R reversion is estimated within an order of magnitude from results in table III. Presumably, if P_{FR} action is not saturated, a change by about 2-fold in the P_{FR} level would give a definite change in germination response. This assumption is based on measurements of many displays to known changes in P_{FR} levels determined by definite irradiances. The 5-fold change in germination between the fourth and twelfth hr on this basis would correspond to a half-time for inactivation of the order of 1 hr as a maximum. Half-times of the order of a few min or 10 hr are surely excluded. A half-time of less than an hr is indicated by Scheibe and Lang's (7) results with lettuce seed at 37° (their table VI).

Combination of a one-half hr half-time for apparent reversion at 35° with a 4-fold change in rate for a 5° shift in temperature, gives a half-time of 500 hr, or about 20 days at 10°, 5 days at 15°, and 1.3 days at 20°. This is in accord with the prechilling effects (layering) at 10° and 15°, but beginning to fail at 20°. The high temperature coefficient for apparent P_{FR} reversion thus appears to be one of the prominent determinative factors for layering. It is important, moreover, that the temperature coefficient for P_{FR} disappearance is much higher than that for P_{FR} action in the germination process.

Literature Cited

1. Downs, R. J., H. A. Borthwick, and A. A. Piringer. 1958. Comparison of incandescent and fluorescent lamps for lengthening photoperiods Proc. Am. Soc. Hort. Sci. 71: 568–78.
2. Ikuma, H. and K. V. Thimann. 1964. Analysis of germination process of lettuce seed by means of temperature and anaerobiosis. Plant Physiol. 39: 756–67.
3. Kinzel, W. 1913-1926. Frost and licht als beeinflussende kräfte bei der samenkeimung. E. Ulmer, Ludwigsburg.
4. Mancinelli, A. L. and H. A. Borthwick. 1964. Photocontrol of germination and phytochrome reaction in dark-germinating seeds of *Lactuca sativa*. Ann. Botan. 28: 9–24.
5. Mancinelli, A. L., Z. Yaniv, and P. Smith. 1967. Phytochrome and seed germination. I. Temperature dependence and relative P_{FR} levels in the germination of dark-germinating tomato seeds. Plant Physiol. 42: 333–37.
6. Roth-Berjerano, N., D. Koller, and M. Negbi. 1966. Mediation of phytochrome in the inductive action of low temperature on dark germination of lettuce seed at supraoptimal temperature. Plant Physiol. 41: 962–64.
7. Scheibe, J. and A. Lang. 1965. Lettuce seed germination: Evidence for a reversible light-induced increase in growth potential and for phytochrome mediation of the low temperature effect. Plant Physiol. 40: 485–92.
8. Stokes, P. 1965. Temperature and seed dormancy. Encyclopedia Plant Physiol. XV-2: 746–803.
9. Tobin, E. M. and W. R. Briggs. 1969. Phytochrome in embryos of *Pinus palustris*. Plant Physiol. 44: 148–50.
10. Toole, E. H., V. K. Toole, S. B. Hendricks, and H. A. Borthwick. 1957. Effect of temperature on germination of light-sensitive seeds. Proc. Intern. Seed Testing Assoc. 22: 1–9.